PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Dhiraj SARDAR *et al.*

Serial No.: 10/543,001

Filed: May 23, 2006

For: METHOD AND APPARATUS FOR DIAGNOSING NEOVASCULARIZED TISSUES

Group Art Unit: 3768

Examiner: Joel F. BRUTUS

Atty. Dkt. No.: UTSJ:041US

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January 4, 2009 — Date

Monica A. De La Paz

DECLARATION OF ANDREW TSIN UNDER 37 C.F.R. §1.132

I, Andrew Tsin, hereby declare as follows:

1. I am a citizen of the United States residing at 8125 Triple Crown, Fair Oaks Ranch, Texas, 78015, USA.

2. I am a Professor of Biochemistry and Cell Biology in the Department of Biology at the University of Texas at San Antonio and an Adjunct Professor of Ophthalmology in the Department of Ophthalmology at the University of Texas Health Science Center in San Antonio I have expertise in the area of ocular biochemistry and cell biology, as well as ophthalmic imaging, as evidenced by my extensive publications in scientific and biophtonics journals in the areas of retinoid metabolism, angiogenic cytokines (for diabetic retinopathy and macular degeneration) and optical properties of healthy and diseased retinas. A copy of my resume is attached as Appendix A.

3. I am one of the inventors of the above-referenced patent application.

4. I have reviewed the Office Action that was mailed on August 4, 2009. I understand that the patent examiner considers the claimed invention to not be patentable because the claimed invention would be obvious to a person of ordinary skill in the field of the invention based on the teachings of the Dreher patent (U.S. Patent 5,303,709) in view of the Hay patent (U.S. Patent 5,632,282), and further in view of the Glaser patent (U.S. Patent 5,767,079), the Larrick patent (U.S. Patent 5,670,151), and/or the Trachtman patent (U.S. Patent 5,002,384). The main reference which the examiner appears to rely on is the Dreher patent.

5. The pending claims concerns methods and apparatuses for diagnosing an ocular disease involving neovascularization. The methods and apparatuses of the invention concern illumination of ocular tissue in a sample holder with a light beam and measurement of particular physical properties of light that is backscattered from the ocular tissue in the sample holder. The physical properties of the backscattered light that are measured include, for example, maximum intensity and/or a polarization shift.

6. The references cited by the patent examiner are distinct from the presently claimed invention because the references do not concern methods or apparatuses that involve measurement of a physical property of backscattered light. The Dreher patent specifically concerns methods and devices for measuring the topography and thickness of the nerve fiber layer that involve measuring the polarization shift of a ***reflected*** probing light. See abstract, FIG. 1, and col. 2, lines 60-68. Thus, rather than measurement of properties of backscattered light, the Dreher patent is ***distinct*** because it concerns measurement of a physical property of reflected light. The Trachtman patent concerns analysis of infrared light ***reflected*** from the patient's cornea and retina for monitoring and training eye position. The Hayes patent concerns methods for diagnosing eye disease that

involve measurement of light ***reflected*** from a patient's retina. The Larrick patent does not concern ophthalmic imaging, but instead concerns methods of controlling hyperproliferative disease of the integument and the eye. The Glaser patent does not concern optical diagnosis, but instead concerns an ophthalmic treatment method employing Transforming Growth Factor β.

7. There are fundamental differences between reflected light and backscattered light. The fundamental differences between these two types of light and matter interactions can be characterized by where the interaction of light and matter occurs, known physical laws which govern the interaction, and the types of interactions between light and matter that occur during these processes. In the most basic physical treatment, reflection is defined as the return of incident electromagnetic radiation (*i.e.*, light) by a surface. This is discussed on page 177 of Optics Source Book. Ed. Parker, Sybil P, 1988 McGraw-Hill, ISBN 0-07-045506-6 (Exhibit A) and page 79 of Optics. Hecht E, 1987 Addison-Wesley Publishing, ISBN 0-201-11609-X (Exhibit B). For an incident beam of light, there exists a number of fundamental laws which describe how the direction of the light is changed by interaction with a "reflective" surface. The law of reflection states that the angle of incidence of light is directly equal to that of the reflected light (see Exhibit A and page 83 of Optics cited above (Exhibit C)). Reflectivity, or a direct measure of the amount of radiation reflected by a material, is dependent upon the angle of incidence, polarization state of incoming radiation, and the electromagnetic properties of the material.

8. In the case of scattered light, of which backscattered light is merely a special case where the angle of scattering is greater than 90 degrees, the interaction between light and matter is approached from a microscopic point of view. Scattering is described as the removal of energy from an incident wave and the subsequent remission of some portion of that

energy. This is discussed on page 293 of Optics cited above (Exhibit D). The angle at which the scattered photon is remitted is highly dependent upon the type of scattering which it undergoes. In complex structures, such as tissue, there are often multiple scattering events from the time the photon enters the material to the time it exits the material. Thus, a photon which is scattered only once will have a different final direction than a photon which is scattered three or four times. This is ultimately responsible for the statistical nature of scattered light in tissues. Due to the optically non-homogenous properties of biological tissues, it is not uncommon to see reflection at the surface, as well as transmission and scattering through biomaterials (see page 3 of Tissue Optics, Tuchin V, 2000 SPIE Press, ISBN: 0-8194-3459-0 (Exhibit E)).

9. In the specific case of the present invention, backscattered light is light that is scattered in such a way that it finds its way back towards the light source and occurs at a range of depths into the tissue.

10. In the Dreher patent, it is specifically stated that the light reflected off of the posterior or anterior surfaces of the eye offers the method of detection (see Column 2 Line 64, Column 3 Line 9, Column 5 Line 1, Column 5 Line 47, Column 10 Line 14). In contrast, the present invention utilizes light which is backscattered throughout the entirety of a tissue layer. More so, it is clearly stated in the Dreher patent that the aim of the device is to determine optic nerve thickness by exploitation of the birefringence properties of that tissue (Column 3 Line 65 – Column 4 Line 20), whereas the present claimed invention seeks to determine degree of a pathological condition (neovascularization as exemplified by angiogenesis of retinal or choroidal capillaries). Thus, there are distinct differences between the methods and apparatuses of the Dreher patent and the present invention.

11. Further, the apparatuses described in the Dreher patent require use of a "corneal polarization compensator." See abstract. The present invention does not involve use of a corneal polarization compensator. Use of this additional component in the context of the present invention would result in loss of information in that it would likely result in loss of some portion of backscattered light. Therefore, one of ordinary skill the field would not be likely to successfully practice the claimed methods of diagnosing an ocular disease involving neovascularization with the apparatuses of the Dreher patent.

12. Given the fundamental differences between the light that is being measured in the Dreher patent (and other cited patents) and the present invention and the requirement for a corneal polarization compensator in the Dreher patent, a person with training in ophthalmic imaging and optics would not have been motivated to modify the Dreher patent or combine it with any of the other references cited by the Examiner to result in the claimed methods and apparatuses. None of the references cited by the patent examiner concern analysis of backscattered light, which is the heart of the present invention.

13. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

January 4, 2010

Date

Andrew Tsin, Ph.D.

Professor

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Andrew Tsin	POSITION TITLE Professor of Biology and Adjunct Professor of Ophthalmology
eRA COMMONS USER NAME ATTSIN	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Dalhousie University, Halifax, N.S., Canada	BSc	1973	Biology
University of Alberta, Edmonton, AB, Canada	MSc	1976	Zoology
University of Alberta, Edmonton, AB, Canada	Ph.D.	1979	Zoology
Baylor College of Medicine, Houston, Tx, USA	Pdf	1979-81	Ophthalmology

A. Positions and Honors:

APPOINTMENTS:

1990-Present Professor, Dept. of Biology, University of Texas at San Antonio (UTSA)
1990-Present Adjunct Professor, Department of Ophthalmology, U of Texas HSC-SA
1991-Present Director, MBRS-SCORE Program, UTSA
2007-Present Director, Center for Research and Training in the Sciences, UTSA
2008-Present Director, Research Center in Minority Institutions, UTSA
2008-Present Associate Dean of Research, College of Sciences, UTSA

FEDERAL GOVERNMENT ADVISORY COMMITTEE:

NSF Reviewer and Panelist, (1987-present)
NIH Reviewer and Site visit Team, (1987-present)
HRSA Reviewer, (1986-1999)
NIH-NIGMS Review Subcommittee, (1999-2003)

B. Selected Peer-Reviewed Publications: (Publications #1-23 published between 1977-1986)

24. Tsin, A.T.C., Flores, J.M., and Rodriguez, K.A. Visual cells, visual pigments and retinoids in the Mongolian Jird. Life Sciences, 41: 2085-2090 (1987).
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C. Research Support:

Ongoing Research Support

FY10/11 Sardar (PI) 9/01/09-8/31/10
Collaborative Research Seed Grant Program
Development of Novel Biomedical Probes Incorporating Rare-Earth Nanocrystals.
The development of novel targeted contrast agents from nanomaterials for medical imaging by a multidisciplinary approach which includes physics, biology and chemistry.
Role: Co-Investigator

5G12RR013646-10 Romo (PI) 08//01/08-07/31/10
NIH/NCRR/RCMI
Cajal Neuroscience Research Center (CNRC)
Establishment of neuroscience research to support core facilities and faculty research at UTSA.
Role: Program Director

5K03 Tsin (PI) 12//06/07-2/28/10
Kronkosky Foundation
A Study in Diabetic Eye Disease – Damage in Diabetic Retinopathy Due to Acrolein and its Mitigation by NBHA.
A study on the effect of acrolein on the levels of oxidized proteins and cytokines in eye and its mitigation by NBHA.
Role: PI

Completed Research Support

POC-RR 1300 Tsin (PI) 12/01/08 – 8/31/09
South Texas Technology Management – Texas Emerging Technology Fund/Office of the Governor of Texas
Adaptive Optics and Retinal Imaging
The goal of this study was to construct a prototype of an adaptive optical system as a diagnostic instrument for retinal imaging
Role: PI

GM 08194-26 Tsin (PI) 8/01/04 -7/31/07
NIH/NIGMS
Pathway of the Cone Visual Cycle
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Role: PI

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TABLE OF CONTENTS

PHYSICAL OPTICS

RICHARD C. LORD

The study of the interaction of electromagnetic waves in the optical range with material systems. The optical range of wavelengths may be taken as the range from about 1 nanometer (4×10^{-8} in.) to about 1 millimeter (0.04 in.). More narrowly, physical optics deals with the relationship between the atomic structure of a system and the manner in which the system affects light sent into it. The chief founder of this branch of science was Michael Faraday, who in 1845 provided the first clue to the electromagnetic nature of light by showing the optical properties of glass could be altered by a magnetic field. SEE FARADAY EFFECT.

The explanation of the absorption, reflection, scattering, polarization, and dispersion of light by a material medium in terms of the properties of the atoms and molecules making up the medium is the objective of physical optics. In the course of seeking this objective, physicists have found that optical investigations are powerful methods of determining the structures of atoms and molecules and of larger systems composed thereof. SEE ABSORPTION OF ELECTROMAGNETIC RADIATION; CRYSTAL OPTICS; DIFFRACTION; DISPERSION (RADIATION); ELECTROMAGNETIC RADIATION; ELECTROOPTICS; INTERFERENCE OF WAVES; LASER; LIGHT; MAGNETOOPTICS; POLARIZED LIGHT; REFLECTION OF ELECTROMAGNETIC RADIATION; REFRACTION OF WAVES; SCATTERING OF ELECTROMAGNETIC RADIATION.

Bibliography. M. Born and E. Wolf, *Principles of Optics*, 6th ed., 1980; F. A. Jenkins, *Fundamentals of Optics*, 4th ed., 1976; R. S. Longhurst, *Geometrical and Physical Optics*, 2d ed., 1974.

REFLECTION OF ELECTROMAGNETIC RADIATION

HERWIG KOGELNIK

The returning or throwing back of electromagnetic radiation such as light, ultraviolet rays, radio waves, or microwaves by a surface upon which the radiation is incident. In general, a reflecting surface is the boundary between two materials of different electromagnetic properties, such as the boundary between air and glass, air and water, or air and metal. Devices designed to reflect radiation are called reflectors or mirrors.

Reflection angle. The simplest reflection laws are those that govern plane waves of radiation. The law of reflection concerns the incident and reflected rays (as in the case of a beam from a flashlight striking a mirror) or, more precisely, the wave normals of the incident and reflected rays and the normal to the reflecting surface all lie in one plane, called the plane of incidence, and that the reflection angle θ_{ref} equals the angle of incidence θ_{inc} as in Eq. (1) [see **Fig. 1**].

$$\theta_{ref} = \theta_{inc} \tag{1}$$

The angles θ_{inc} and θ_{ref} are measured between the surface normal and the incident and reflected rays, respectively. The surface (in the above example, that of the mirror) is assumed to be smooth, with surface irregularities small compared to the wavelength of the radiation. This results in so-called specular reflection. In contrast, when the surface is rough, the reflection is diffuse. An example of this is the diffuse scattering of light from a screen or from a white wall where light is returned through a whole range of different angles.

Reflectivity. The reflectivity of a surface is a measure of the amount of reflected radiation. It is defined as the ratio of the intensities of the reflected and incident radiation. The reflectivity depends on the angle of incidence, the polarization of the radiation, and the electromagnetic properties of the materials forming the boundary surface. These properties usually change with the wavelength of the radiation. Reflecting materials are divided into two groups: transparent materials and opaque conducting materials. Radiation penetrating a transparent material propagates essentially unattenuated, while radiation penetrating a conducting material is heavily attenuated. Transparent materials are also called dielectrics. In the wavelength range of visible light, typical dielectrics are glass, quartz, and water. Conducting materials are usually metals such as

OPTICS

SECOND EDITION

EUGENE HECHT
Adelphi University

With Contributions by Alfred Zajac

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h that $\lambda_{0j}\nu_{0j} = c$. This formulation is a consider-
actical improvement over the Cauchy equation.
:hat where $\lambda \gg \lambda_{0j}$, Cauchy's equation is an
imation of Sellmeier's. *Hint:* write the above
ion with only the first term in the sum; expand
e binomial theorem; take the square root of n^2
pand again.

If an ultraviolet photon is to dissociate the oxy-
id carbon atoms in the carbon monoxide
le, it must provide 11 eV of energy. What is the
m frequency of the appropriate radiation?

4 THE PROPAGATION OF LIGHT

4.1 INTRODUCTION

We now consider a number of phenomena related to the propagation of light and its interaction with material media. In particular, we shall study the characteristics of lightwaves as they progress through various substances, crossing interfaces, and being reflected and refracted in the process. For the most part, we shall envision light as a classical electromagnetic wave whose velocity through any medium is dependent upon that material's electric and magnetic properties. It is an intriguing fact that many of the basic principles of optics are predicated on the wave aspects of light but are completely independent of the exact nature of the wave. As we shall see, this accounts for the longevity of *Huygens's principle*, which has served in turn to describe mechanical aether waves, electromagnetic waves, and now, after three hundred years, applies to quantum optics.

Suppose, for the moment, that a wave impinges on the interface separating two different media (e.g., a piece of glass in air). As we know from our everyday experiences, a portion of the incident flux density will be diverted back in the form of a *reflected wave*, while the remainder will be transmitted across the boundary as a *refracted wave*. On a submicroscopic scale we can envision an assemblage of atoms that scatter the incident radiant energy. The manner in which these emitted light wavelets superimpose and combine with each other will depend on the spatial distribution of the scattering atoms. As we know from the previous chapter, the scattering process is responsible for the *index of refraction*, as well as the resultant *reflected* and *refracted* waves. This atomistic description is quite satisfying conceptually, even though it is not a simple matter to treat analytically. It should, however, be kept in mind even when applying macroscopic techniques, as indeed we shall later on.

We now seek to determine the general principles governing or at least describing the propagation, reflection, and refraction of light. In principle it should be possible to trace the progress of radiant energy through any system by applying Maxwell's equations and the associated boundary conditions. In practice, however, this is often an impractical if not an impossible task (see Section 10.1). So we shall take a somewhat different route, stopping, when appropriate, to verify that our results are in accord with electromagnetic theory.

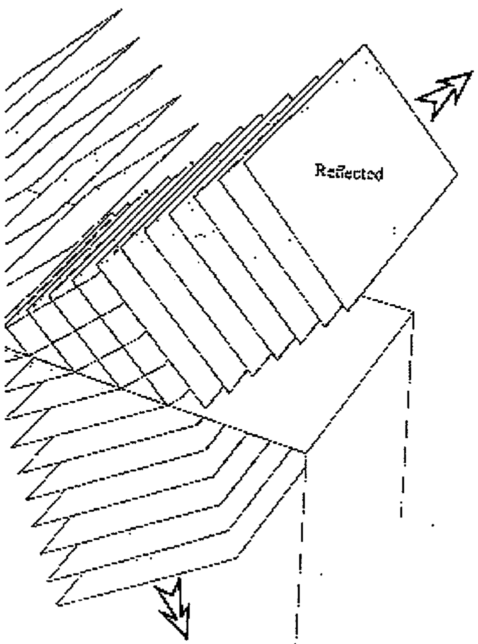
4.2 THE LAWS OF REFLECTION AND REFRACTION

4.2.1 Huygens's Principle

Recall that a wavefront is a surface over which an optical disturbance has a constant phase. As an illustration, Fig. 4.1 shows a small portion of a spherical wavefront Σ emanating from a monochromatic point source S in a homogeneous medium. Clearly, if the radius of the wavefront as shown is r, at some later time t it will simply be $(r + vt)$, where v is the phase velocity of the wave.

EXHIBIT C

suppose the incident wave comes in at some ıgle, as indicated in Fig. 4.5. Clearly, it sweeps ıe interface again, essentially splitting into two ne reflected and one refracted. Let's follow the of a typical front in Fig. 4.6, envisioning the as if it were a series of snapshots taken in e intervals of time τ. Start when Σ_i makes ith the interface at point a. At that point, both cted and transmitted wavefronts begin, so a, s on both fronts, can be taken as a source of ıpwardly emitted wavelet traveling at a speed downwardly emitted wavelet traveling at a Now focus on another point, say, b on Σ_i. time t_1 the plane Σ_i will have moved a distance ident medium of $v_i t_1$, so that b then corre- b'. Presumably, two wavelets will then propa- rom b' into the incident and transmitting ntributing to the reflected, Σ_r', and transmit- vefronts. These wavelets are shown here after where $\tau = t_1 + t_2$. The rest of the diagram



ction and transmission of plane waves.

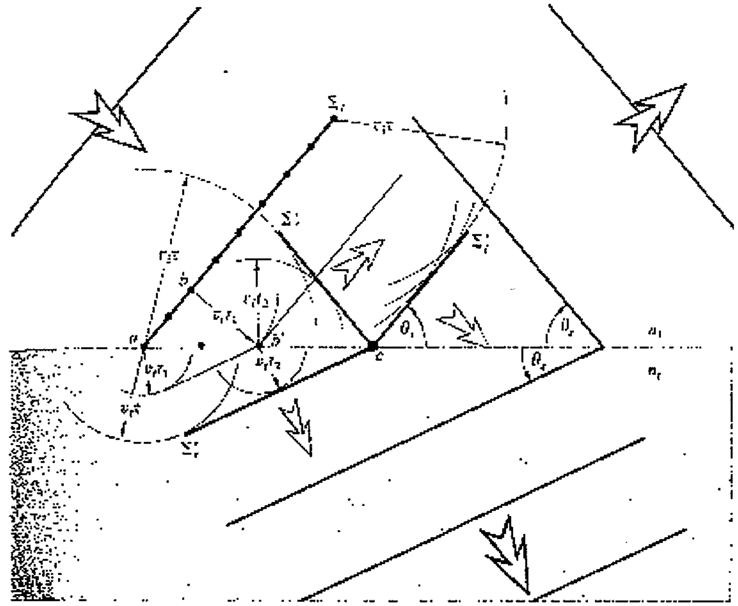


Figure 4.6 Reflection and transmission at an interface via Huygens's principle.

should be self-explanatory. Figure 4.7 is a somewhat simplified version in which θ_i, θ_r, and θ_t, as before, are the angles of *incidence*, *reflection*, and *transmission* (or *refraction*), respectively. Notice that

$$\frac{\sin\theta_i}{\overline{BD}} = \frac{\sin\theta_r}{\overline{AC}} = \frac{\sin\theta_t}{\overline{AE}} = \frac{1}{\overline{AD}}. \tag{4.1}$$

By comparison with Fig. 4.6, it should be evident that

$$\overline{BD} = v_i t, \qquad \overline{AC} = v_i t, \qquad \overline{AE} = v_t t,$$

so substituting into Eq. (4.1) and canceling t, we have

$$\frac{\sin\theta_i}{v_i} = \frac{\sin\theta_r}{v_i} = \frac{\sin\theta_t}{v_t}. \tag{4.2}$$

It follows from the first two terms that **the angle of incidence equals the angle of reflection**, that is,

$$\theta_i = \theta_r. \tag{4.3}$$

Known as the **law of reflection**, it first appeared in the book entitled *Catoptrics*, which was purported to have been written by Euclid.

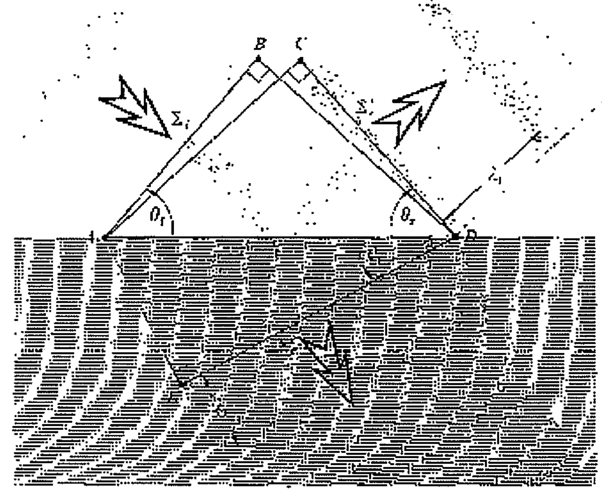
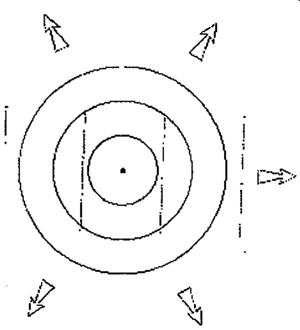


Figure 4.7 Reflected and transmitted wavefronts at a given instant.

TERING AND POLARIZATION

Introduction to Scattering

in to understand many apparently unrelated
: in terms of differing aspects of the same
tomic processes, and so we again return to
1. When an electromagnetic wave impinges
: or molecule it interacts with the bound
ud, imparting energy to the atom. The effect
:red as if the lowest energy or ground state
1 were set into vibration. The oscillatory
f the electron cloud is equal to the driving
, that is, the frequency of the harmonic
e lightwave. The amplitude of the oscilla-
elatively large only when ν is in the vicinity
ant frequency of the atom. In fact, at reso-
employ the simple description of the atom
g in its ground state; upon absorbing a
ng the resonating frequency), it makes the
an excited state. In dense media, the atom
ly return to its ground state, having dissi-
ss energy thermally. In rarefied gases the
nerally make the downward transition by
oton, an effect known as *resonance radiation*.
cies below or above resonance, the elec-
g with respect to the nucleus may be regar-
ing electric dipoles, and as such they will



ering of a spherical wavelet.

generally reradiate electromagnetic energy at a frequency coinciding with that of the incident light. This nonresonant emission propagates out in the dipole radiation pattern of Fig. 3.21. *The removal of energy from an incident wave and the subsequent reemission of some portion of that energy is known as scattering* (Fig. 8.33). It is the underlying physical mechanism operative in reflection, refraction, and diffraction; the scattering process is fundamental indeed.

In addition to electron-oscillators, which generally have resonances in the ultraviolet, there are atomic-oscillators, which correspond to the vibration of the constituent atoms within a molecule. Because of their large masses, atomic-oscillators usually have resonances in the infrared. Moreover, they have relatively small vibrational amplitudes and are therefore of little concern here.

The amplitude of an oscillator, and thus the amount of energy removed from the incident wave, increases as the frequency of the wave approaches a natural frequency of the atom. For low-density gases, in which atomic interactions are negligible, absorption will be insignificant, and the reradiated or scattered wave will carry off increasingly more energy as the driving frequency approaches a resonance. This results in some rather interesting effects when the atom's natural frequencies are in the ultraviolet and the incident wave is in the visible region. In that case, as the frequency of the incoming light increases, more and more of it will be elastically scattered. As an example, imagine that you are outside on a bright clear morning. The sky is a brilliant blue, and you are surrounded, even inundated, with blue light. Sunlight streaming into the atmosphere from one direction is scattered in all directions by the air molecules. Without an atmosphere, the daytime sky would be as black as the void of space, a point well made in the Apollo lunar photographs (Fig. 8.34). You would then see only light that shone directly at you. With an atmosphere, the red end of the spectrum is, for the most part, undeviated, whereas the blue or high-frequency end is substantially scattered. This high-frequency scattered light reaches the observer from many directions, making the entire sky appear bright and blue (Fig. 8.35). When the Sun is very low in the sky, its rays pass through a great thickness of air. The

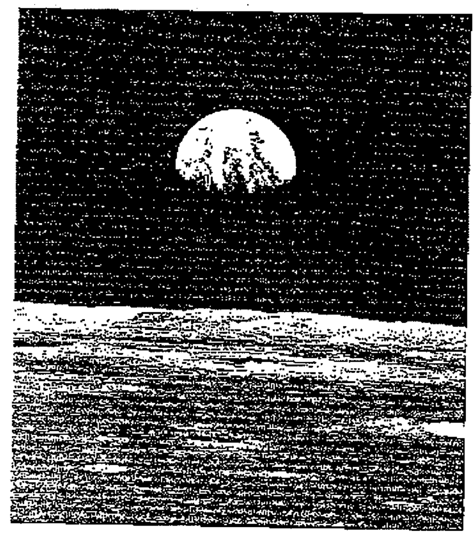


Figure 8.34 A half-Earth hanging in the black Moon sky. (Photo courtesy NASA)

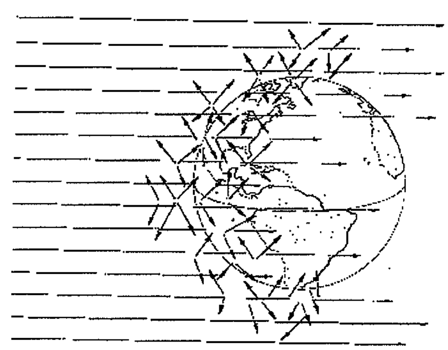


Figure 8.35 Scattering of sky light.

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TISSUE OPTICS

Light Scattering Methods and Instruments for Medical Diagnosis

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To my grandkids,

Dasha, Zhenya, and Stepa

OPTICAL PROPERTIES OF TISSUES WITH STRONG (MULTIPLE) SCATTERING

This first chapter introduces the problem of light (laser beams) transport within strongly (multiple) scattering tissues, such as skin, breast, brain, and vessel wall. Basic principles and theoretical descriptions using radiation transfer theory or Monte Carlo (MC) simulation are considered. Methods for solving the inverse problem of finding tissue optical parameters and their advantages and drawbacks are analyzed. Since contrast of optical images always depends on the optical properties of the tissues under investigation, the specifics of controlling tissue optical parameters are considered. The propagation of short pulses and photon-density diffusion waves in scattering and absorbing media is analyzed and the prospects of these methods for tissue spectroscopy and tomography are discussed. Polarization phenomena and optothermal and optoacoustic interactions in strongly scattering tissues are described. A discrete-particle model of soft tissue is presented. The design and characterization of tissue-like phantoms for optical diagnostics are described.

1.1 PROPAGATION OF CONTINUOUS WAVE LIGHT IN TISSUES

1.1.1 Basic principles and major absorbers

Biological tissues are optically inhomogeneous and absorbing media whose average refractive index is higher than that of air. This is responsible for partial reflection of the radiation at the tissue/air interface (Fresnel reflection), while the remaining part penetrates the tissue. Multiple scattering and absorption are responsible for laser beam broadening and eventual decay as it travels through a tissue, whereas bulk scattering is a major cause of the dispersion of a large fraction of radiation in the backward direction. Cellular organelles such as mitochondria are the main scatterers in various tissues.

Absorbed light is converted to heat or radiated in the form of fluorescence, it is also consumed in photobiochemical reactions. The absorption spectrum depends on the type of predominant absorption centers and water content of tissues (see Figures 1.1-1.4). Absolute values of absorption coefficients for typical tissues lie in the range 10^{-2} to 10^{4} cm^{-1}.[1–4,6,9–15,28,29,31,37–42,56,57,72,86–91] In the ultraviolet (UV) and infrared (IR) ($\lambda \geq 2\ \mu m$) spectral regions, light is readily absorbed,